TRIGGERS FOCUS GROUP
RECOMMENDATION #8

Toxicity Tests with Controls that did not Meet US EPA Test Acceptability Criteria for NPDES Testing

September 19, 2006

OBJECTIVE: The objective of this requirement is to provide guidance regarding how to interpret toxicity data from tests with Lab Controls that did not meet the US EPA test acceptability criteria.

PROBLEM STATEMENT: The agricultural community has formed Coalitions for their watersheds so that they have a logical geographical boundary for monitoring, as well as a group of growers to pay for the monitoring. The watersheds range from relatively small (e.g., a water district with 3-5 samples per sampling event) to very large (e.g., Sacramento Valley with ~20 sites per sampling event); most Coalitions are monitoring anywhere from 8 to 12 events per year. Toxicity testing for these Coalitions includes acute *Ceriodaphnia*, acute fathead minnow, and the chronic *Selenastrum* tests.

The following test acceptability criteria have been established for NPDES testing in the EPA test guidance manuals:

Test	EPA Manual Test Acceptability Criteria (TAC)
Acute *Ceriodaphnia*	≥90% survival in the Control treatment
Acute Fathead Minnow	≥90% survival in the Control treatment
Chronic *Selenastrum*	mean cell density of 200,000 cells/mL (when tested w/o EDTA) and CV <20% at the Control treatment.

For NPDES testing, the manual indicates that if the test acceptability criteria are not met, the test must be repeated with a newly collected sample. The EPA acknowledges that 1 in 20 toxicity tests may not meet test acceptability criteria based on statistical protocols alone.

The initial statement made in both the acute and chronic manuals (Section 1.1) indicate, "This manual describes acute (or chronic) toxicity tests for use in the National Pollutant Discharge Elimination System (NPDES) Permits Program". The test conditions and test acceptability criteria within these manuals were established with this in mind. However, the logistical and regulatory framework inherent to the ILP monitoring is very different from most (if not all) NPDES testing situations. For example:

- Much is riding on each NPDES sample due to the limited frequency of monitoring when compared to a more wide-scale monitoring program (e.g., ILP monitoring); and
- Re-collection of a sample for a test that does not meet US EPA TAC would typically occur at a single "point source" discharge in most NPDES cases, whereas re-collection of samples under an ILP monitoring program could require the re-collection of samples from multiple sites.

Given this, the fundamental question before the TIC is: how should we deal with tests whose Control treatment(s) do not meet the US EPA TAC?

In answering this question, a new set of important "follow-up" questions and/or issues becomes apparent. In order to answer the more fundamental questions, it will be important to consider these other questions:

- Arguably, the most important consideration in determining how to deal with a Control treatment that failed to meet TAC is the question of whether or not the Control treatment results truly preclude a definitive determination as to the presence or absence of toxicity. The answer to that is often very straightforward: No. It is important to remember that if a sample is toxic, there must be a statistically significant negative effect; if there is no such negative effect, then ***by definition***, there is no toxicity assuming that the testing was properly performed following the EPA method.

    - For the **acute survival tests**, the water samples often exhibit 90 -100% survival. In these cases, the absence of any statistically significant negative effect in the water sample is in-and-of-itself (and by definition) a definitive indication that there is no toxicity.

    - For the **chronic algal growth test**, the water samples often exhibit algal growth that is markedly greater than the Control treatment response, again providing a definitive indication that there is no toxicity.

The ILP that has numerous scientists of national caliber that help evaluate and interpret monitoring and toxicity testing data. This wealth of scientific expertise allows us to make decisions and evaluate results on the basis of Good Science and Best Professional Judgement.

RECOMMENDATION: In taking into account the above questions, issues, and examples, the resolution of "how to deal with a test (or tests) for which the accompanying Control treatment survival response, growth response, and/or inter-replicate variability does not meet the US EPA TAC" seems best addressed via a flow-chart type decision-making structure.

The paradigm that **if a sample is toxic, there must be some degree of impairment; if there is no impairment, then *by definition*, there is no toxicity** should be a fundamental element of this decision-making. It is proposed that Best Professional Judgment be used for interpreting toxicity tests that do not meet the US EPA TAC. At a minimum, the proposed approach is to include the following elements, which are to be addressed in the Coalition QAPP:

Decision Step 1: If the Control treatment meets all US EPA TAC, then proceed to statistical analyses for determination of the presence of statically significant reductions in organism survival or algal growth. For samples that exhibit toxicity, the follow-up requirements in the ILP MRP must be followed.

Proposed Decision Step 2a: If an acute test of a water sample exhibits 90-100% survival, and the program completeness standard for the test is met (e.g., ≥90% of testing performed successfully to meet SWAMP compatibility), no further testing is required; test result should be "flagged" to denote 90% survival in the Control treatment. If an acute test of a water sample exhibits 90-100% survival, and the program completeness standard for the test is not met, then a re-test must

be initiated within 24 hours of the observation of a Control treatment with <90% survival. In this case, both the original test results and the re-test results must be reported by the Coalition; the re-test results should be flagged to note that the re-test was initiated outside of the holding time limit. New samples must be collected if the re-test does not meet US EPA TAC.

Proposed Decision Step 2b: If an algal test of a water sample exhibits an algal cell density that is greater than the algal cell density at the Control treatment, and the Control test does not meet the US EPA TAC, it is proposed that instead of the one-tailed statistical tests (which ask only if the test response for a sample is "less" than the Control), a 2-tailed statistical test be performed. If the results of that test indicate that the algal growth in the water sample is significantly greater than the Control treatment, and the program completeness standard for the test is met, then the sample should be determined to be not toxic; test result should be "flagged" to denote <200,000 cells/ml or CV>20% survival in the Control treatment. If the program completeness standard for the test is not met, then a re-test must be initiated within 24 hours of the termination of the initial algal test. In this case, both the original test results and the re-test results must be reported by the Coalition; the re-test results should be flagged to note that the re-test was initiated outside of the holding time limit. New samples must be collected if the re-test does not meet US EPA TAC.

Proposed Decision Step 3: If a Control treatment does not meet US EPA TAC, the associated ambient water sample(s) have <90% survival (for an acute toxicity test) or the algal growth is less than the Control, and the sample is not toxic, then Best Professional Judgment must be used to evaluate the data. It is expected that the Regional Board will be notified within 1 business day of the observation of the results in question so that an agreement can be reached regarding how to proceed.

Proposed Decision Step 4: If either an acute test or an algal test does not meet the US EPA TAC and statistical analyses indicates that the sample is toxic, then the data must be assessed against the triggers in the MRP (e.g., samples with significant toxicity must be re-sampled, samples with ≥50% reduction in organism response requires a TIE, and samples with 100% mortality require a dilution series).

The reporting of data that do not meet US EPA TAC must also include an assessment from the laboratory as to what may have caused the test control performance issue, what the laboratory is doing to prevent this from happening again in the future, a comparison of the data against the EPA test performance measures, and a comparison of the data against the ILP required completeness criteria in the Coalition's QAPP.